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EFFECT OF BUTACHLOR (2-CHLORO-2', 6'-DIETHYL-N-) BUTOXYMETHYL(-ACETANILIDE) ON THE BASIPETAL TRANSPORT OF EXOGENOUS INDOLE-3-ACETIC ACID IN MAIZE SEEDLINGS

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Abstract

In maize seedlings grown in a culture solution, the basipetal transport of indole-3-acetic acid labelled on the carboxyl group, which had been located on the leaf surface, was delayed by butachlor (2-chloro-2', 6'-diethyl-N-)butoxymethyl(-acetanilide) in a concentration of 20 ppm. Inhibition of the transport led to the accumulation of indole-3-acetic acid in the region of the accessory roots.

Introduction

Butachlor, a chloracetanilide, is a selective herbicide which is employed primarily pre-emergently against mono- and dicotyledonous weeds in rice, cotton and soya cultures, but which can also be used for the treatment of maize, sunflower and sugar-beet.

The α-chloracetanilides are germination-inhibiting herbidides, but their herbicidal effect is not unambiguously a consequence of the germination-inhibition. In many plants the herbicide inhibits not the germination, but the normal growth following the germination. The growth-inhibiting effect extends to the cell-division and elongation (HICKEY, et al. 1974; DHILLON and ANDERSON, 1972; ESHEL, 1969; EDMONDSON, 1969; KEELY, 1972).

The growth-inhibiting effect is in all probability closely connected with the inhibitory effect of the α-chloracetanilides on protein synthesis (JAWORSKI, 1969; MORELAND, 1969; DUKE, 1967, 1975).

Our observations on maize plants indicate that butachlor induces morphological changes primarily in the roots, in so far as it decreases the number of second-order branchings and increases the number of adventitious roots formed at the first internodes. Since the increase in the number of adventitious roots is indicative that the change takes place in the auxin household of the plants on herbicide treatment, we carried out experiments to study the changes in the transport and metabolism of indole-3-acetic acid (IAA) in the organs of the treated maize plants. In the present paper we deal with the effect of butachlor on auxin transport.

Materials and Methods

As experimental plants, 12-day-old seedlings of *Zea mays* L. cvar. *Keszthelyi* SC. were used, which had been grown in a four-fold diluted Knop culture solution (pH 6.8) after pregermination for 3 days in a thermostat at 25 °C. Five seedlings were placed in each of the vessels, which contained 450 ml culture solution. The experiments were carried out under controlled conditions in a "CONVIRON" growth chamber, with 14 hours of daylight, a daytime temperature of 24 °C, a nighttime temperature of 18 °C, and a relative humidity of 60%.

The radioactive butachlor was synthetized in the Isotope Laboratory of the Biological Research Center of the Hungarian Academy of Sciences. The specific activity of the preparation was 0.61 Ci/mmole.

The correlation between the herbicide effect and the IAA transport was investigated under the following experimental conditions.

1. Uptake of ^{14}C-butachlor. At the age of 12 days, the seedlings were placed in a four-fold diluted Knop solution containing 20 ppm unlabelled butachlor. A total amount of 50 μci of butachlor was added to the culture solution in each culture vessel. In the course of the sampling, the plants were placed in an electric drying press and the autoradiograms were prepared on "Forte Medifort R" medical X-ray film with an exposure time of one week. The same procedure was employed for every sample.
2. Uptake of ^{14}C-IAA. In the form of 0.1 ml of a 50% ethanolic solution, and in a total amount of 0.5 μCi per plant, ^{14}C-IAA labelled on the carboxyl group was transferred to the third, youngest leaf of the 12-day-old, three-leaved seedlings growing in the Knop culture solution.
3. Transport of ^{14}C-IAA with the simultaneous use of hormone and butachlor. A four-fold diluted Knop solution containing 20 ppm butachlor was prepared, and the 12-day-old seedlings were treated with ^{14}C-IAA as described in point 2, in parallel with their transfer to the herbicide-containing culture solution.
4. Transport of ^{14}C-IAA in plants pretreated with butachlor. The procedure was similar to that described in point 3, but the labelled IAA was transferred to the leaf 24 hours after the herbicide treatment.

Results and discussion

The transport and translocation of the exogenous IAA were studied with maize plants treated with butachlor via the roots. The use of labelled butachlor was of benefit for the correct selection of the intake time of the IAA into the plants and for the investigation of the translocation of the butachlor. In the experiments the transports of the two compounds were studied when employed separately and in combination.

1. Uptake of ^{14}C-butachlor

The uptake of the labelled herbicide via the root can still barely be perceived in the sample taken 2 hours after the treatment; merely the grain displays a slight labelling, which is probably connected with the higher oil content. Even 12 hours later only the roots contain labelling (Fig. 1). The translocation of the butachlor is relatively slow; in practice, the butachlor does not enter the aboveground parts.

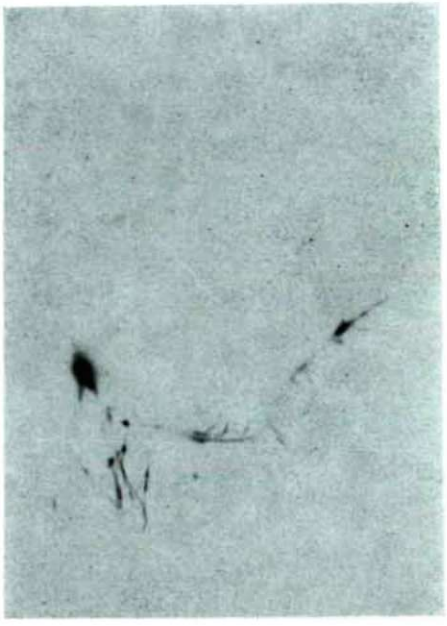

Fig. 1. Transport of ^{14}C-butachlor in 12-day-old maize seedlings. Distribution of radioactivity in the seedling 2 hours after treatment.
The bulk of the radioctivity is accumulated in the grain, with a small amount in the root. The butachlor is not translocated into the aboveground parts later either. Activity of butachlor added to culture solution: 1.1 μci/ml. The autoradiagram was prepared on, "Forte Medifort R" film. Exposure time: 1 week.

2. Uptake of ^{14}C-IAA

Two hours after the treatment, the labelled IAA taken up via the leaf is translocated throughout the entire length of the maize seedling. And enhanced radioactivity compared to the untreated specimens can not be found in the zone of the adventitious roots (Fig. 2).

3. Combined uptake and translocation of IAA and butachlor when applied at the same and at different times

In the experiments, unlabelled butachlor was added to the culture solution, and 1-^{14}C-IAA was transferred to the leaf. If the two compounds were applied simultaneously, the auxin transport is unimpeded in the 2-hour sample (when the herbicide uptake is still not appreciable). 6 and 12 hours after the treatment, when the butachlor has already accumulated in the roots, the radioactivity is accumulated in the adventitious root zone and above it (Fig. 3).

If butachlor pretreatment is employed before the addition of labelled IAA, in the 2-hour sample the translocation of auxin is inhibited compared to that of the plant maintained in herbicide-free culture solution, and the roots barely display radioactivity. In the 6 and 12-hour samples a significant labelling accumulation can be observed in the nodes above the adventitious region. This originates from inhibition of the basipetal transport of the IAA (Fig. 4).

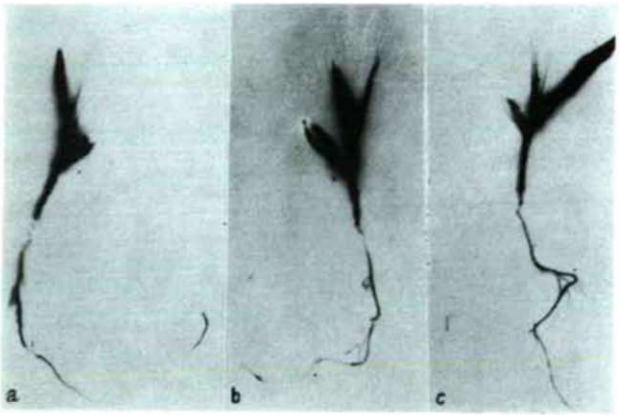

Fig. 2. Translocation of carboxyl-^{14}C-IAA in 12-day-old maize seedlings. Distribution of radioactivity in the plants (a) 2, (b) 6 and (c) 12 hours after treatment. Total activity on the third leaf: 0.5 μci per plant. The autoradiagrams were prepared on, "Forte Medifort R" film with an exposure time of one week.

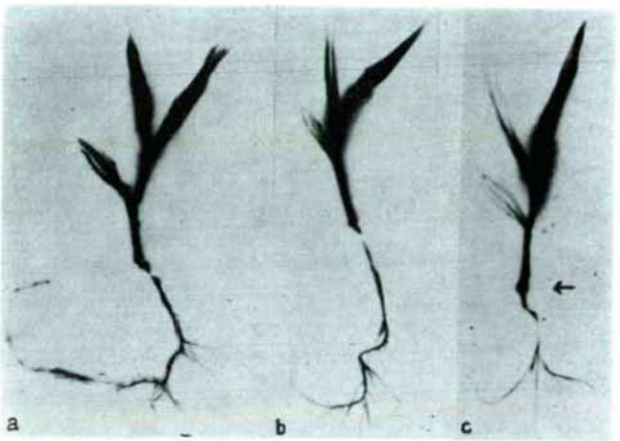

Fig. 3. Translocation of carboxyl-^{14}C-IAA in 12-day-old maize seedlings treated with butachlor. Distribution of radioactivity in the plants (a) 2, (b) 6 and (c) 12 hours after uptake. The butachlor and labelled IAA were added simultaneously to the culture solution. An extensive accumulation can be observed in the stem at the sites marked with arrows.

On the basis of the results it is concluded that butachlor slows down the basipetal transport of the exogenous IAA from the shoot towards the root; this may occur by means of direct inhibition, but it may also be the result of secondary processes.

Since one of the most important preconditions of the initiation of the adventitious roots (together with other factors) is a high concentration of IAA at the appropriate sites, it seems certain that the butachlor increases the possibility of the formation of adventitious roots by inhibiting the transport of IAA.

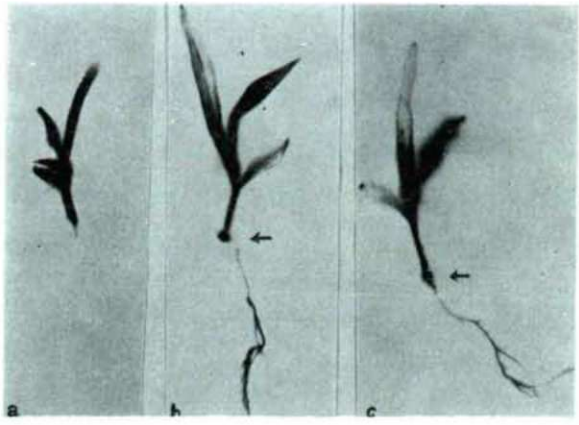

Fig. 4. Translocation of carboxyl-^{14}C-IAA in 12-day-old maize seedlings pretreated with butachlor-Distribution of radioactivity in the plants (a) 2, (b) 6 and (c) 12 hours after uptake. ^{14}C-IAA was transferred to the third leaf 24 hours after treatment with butachlor. Explanation as in Fig. 3.

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